

What is claimed is:

1. A method for making a transformed plant that selectively increases production of GABA in response to a signal, comprising:

5 incorporating into a plant's genome a DNA construct comprising a non-constitutive promoter operably linked to a polynucleotide that encodes a functional plant GAD enzyme, to provide a transformed plant;

wherein the transformed plant expresses the polynucleotide in response to a signal.

10 2. The method according to claim 1, wherein the promoter is selected from the group consisting of a tissue preferred promoter, a tissue specific promoter, a cell type specific promoter and an inducible promoter.

15 3. The method according to claim 2, wherein the promoter is a tissue preferred promoter.

4. The method according to claim 2, wherein the promoter is a tissue specific promoter.

20 5. The method according to claim 2, wherein the promoter is a cell type specific promoter.

6. The method according to claim 2, wherein the promoter is an inducible promoter.

25 7. The method according to claim 6, wherein the inducible promoter is responsive to a signal selected from the group consisting of mechanical shock, heat, cold, salt, flooding, drought, wounding, anoxia, pathogens, ultraviolet-B, nutritional deprivation, a flowering signal, a fruiting signal, cell specialization and combinations thereof.

8. The method according to claim 1, wherein the GAD enzyme comprises an amino acid sequence selected from the group consisting of the sequence set forth in SEQ ID NO: 2; the sequence set forth in SEQ ID NO: 4; the sequence set forth in SEQ ID NO: 6; the sequence set forth in SEQ ID NO: 8; the sequence set forth in SEQ ID NO: 10; the sequence set forth in SEQ ID NO: 12; the sequence set forth in SEQ ID NO: 14; the sequence set forth in SEQ ID NO: 16; the sequence set forth in SEQ ID NO: 18 and a sequence having at least about 60% identity thereto that is effective to catalyze a reaction of glutamic acid to GABA.

10 9. The method according to claim 1, wherein the GAD enzyme is a modified GAD that does not include a functional autoinhibitory calmodulin-binding domain.

15 10. The method according to claim 1, wherein the transformed plant produces GAD enzymes in response to the signal at a rate greater than the rate at which GAD enzymes are produced by a non-transformed plant of the same species under the same conditions.

20 11. The method according to claim 1, wherein the target plant is selected from the group consisting of duckweed, rice, wheat, barley, rye, corn, Bermuda grass, Blue grass, fescue, rapeseed, potato, carrot, sweet potato, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, 25 pineapple, avocado, papaya, mango, banana, soybean, bush beans, tobacco, tomato, green pepper, sorghum and sugarcane.

20 12. The method according to claim 1, wherein the polynucleotide is selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1; the nucleotide sequence of SEQ ID NO: 3; the nucleotide sequence of SEQ ID NO: 5; the nucleotide sequence of SEQ ID NO: 7; the nucleotide sequence of SEQ ID

NO: 9; the nucleotide sequence of SEQ ID NO: 11; the nucleotide sequence of SEQ ID NO: 13; the nucleotide sequence of SEQ ID NO: 15; the nucleotide sequence of SEQ ID NO: 17 and sequences that hybridize thereto under moderately stringent conditions.

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13. The method of Claim 1, wherein said incorporating comprises:

- (i) transforming a cell, tissue or organ from a host plant with the DNA construct;
- (ii) selecting a transformed cell, cell callus, somatic embryo, or seed which contains the DNA construct;
- (iii) regenerating a whole plant from the selected transformed cell, cell callus, somatic embryo, or seed; and
- (iv) selecting a regenerated whole plant that expresses the polynucleotide.

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14. A transformed plant obtained according to the method of claim 1 or progeny thereof.

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15. The transformed plant according to claim 14, wherein the DNA construct is incorporated into the plant in a homozygous state.

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16. A DNA construct comprising a non-constitutive promoter operably linked to a polynucleotide that encodes a GAD enzyme; wherein the promoter regulates expression of the polynucleotide in a host cell in response to a signal.

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17. The DNA construct in accordance with claim 16, wherein the GAD enzyme comprises an amino acid sequence selected from the group consisting of the sequence set forth in SEQ ID NO: 2; the sequence set forth in SEQ ID NO: 4; the sequence set forth in SEQ ID NO: 6; the sequence set forth in SEQ ID NO: 8; the sequence set forth in SEQ ID NO: 10; the sequence set forth in SEQ ID NO: 12; the sequence set forth in SEQ ID NO: 14; the sequence set forth in SEQ ID NO: 16; the

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sequence set forth in SEQ ID NO: 18 and a sequence having at least about 60% identity thereto that is effective to catalyze a reaction of glutamic acid to GABA.

18. The DNA construct according to claim 16, wherein the  
5 polynucleotide comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1; the nucleotide sequence of SEQ ID NO: 3; the nucleotide sequence of SEQ ID NO: 5; the nucleotide sequence of SEQ ID NO: 7; the nucleotide sequence of SEQ ID NO: 9; the nucleotide sequence of SEQ ID NO: 11; the nucleotide sequence of SEQ ID NO: 13; the nucleotide  
10 sequence of SEQ ID NO: 15; the nucleotide sequence of SEQ ID NO: 17 and sequences that hybridize thereto under moderately stringent conditions.

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19. The DNA construct according to claim 16, wherein the promoter is a tissue specific plant promoter.

20. The DNA construct according to claim 16, wherein the promoter is an inducible plant promoter.

21. A vector useful for transforming a cell, said vector comprising the  
20 DNA construct according to claim 16.

22. A plant transformed with the vector of claim 21, or progeny thereof, wherein the plant expresses the polynucleotide in response to a signal.

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23. The plant according to claim 22, the plant being selected from the group consisting of duckweed, rice, wheat, barley, rye, corn, Bermuda grass, Blue grass, fescue, rapeseed, potato, carrot, sweet potato, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, zucchini, cucumber, apple, pear, quince,  
30 melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry,

blackberry, pineapple, avocado, papaya, mango, banana, soybean, bush beans, tobacco, tomato, green pepper, sorghum and sugarcane.

24. A cell having incorporated therein a foreign gene comprising a non-  
5 constitutive promoter operably linked to a polynucleotide encoding a functional  
plant GAD enzyme.

25. The cell according to claim 24, wherein the enzyme comprises an  
amino acid sequence selected from the group consisting of the sequence set forth in  
10 SEQ ID NO: 2; the sequence set forth in SEQ ID NO: 4; the sequence set forth in  
SEQ ID NO: 6; the sequence set forth in SEQ ID NO: 8; the sequence set forth in  
SEQ ID NO: 10; the sequence set forth in SEQ ID NO: 12; the sequence set forth  
in SEQ ID NO: 14; the sequence set forth in SEQ ID NO: 16; the sequence set  
forth in SEQ ID NO: 18 and a sequence having at least about 60% identity thereto.  
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26. The cell according to claim 24, wherein the cell is a plant cell.

27. A plant having incorporated therein a foreign gene comprising a  
non-constitutive promoter operably linked to a polynucleotide encoding a  
20 functional plant GAD enzyme.

28. The plant according to claim 27, wherein the enzyme comprises an  
amino acid sequence selected from the group consisting of the sequence set forth in  
SEQ ID NO: 2; the sequence set forth in SEQ ID NO: 4; the sequence set forth in  
25 SEQ ID NO: 6; the sequence set forth in SEQ ID NO: 8; the sequence set forth in  
SEQ ID NO: 10; the sequence set forth in SEQ ID NO: 12; the sequence set forth  
in SEQ ID NO: 14; the sequence set forth in SEQ ID NO: 16; the sequence set  
forth in SEQ ID NO: 18 and a sequence having at least about 60% identity thereto

29. A chimeric polynucleotide causing increased GABA production in a plant cell transformed with the chimeric polynucleotide, comprising:

a regulatory sequence comprising a non-constitutive promoter; and  
a nucleic-acid fragment encoding a functional plant GAD enzyme.

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30. The chimeric polynucleotide in accordance with claim 29, wherein the nucleic acid fragment comprises a member selected from the group consisting of:

- (i) a nucleic acid fragment encoding an enzyme having the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16 or 18;
- 10 (ii) a nucleic acid fragment encoding an enzyme having an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16 or 18, encompassing amino acid substitutions, additions and deletions that do not eliminate the function of the enzyme;
- 15 (iii) a nucleic acid fragment of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 or 17; and
- (iv) a nucleic acid fragment of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 or 17, encompassing base changes that do not eliminate the function of the encoded enzyme.

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31. A method for making a transformed plant, comprising:

providing a vector comprising a constitutive promoter operably linked to a polynucleotide that encodes a plant GAD enzyme;

transforming one or more plants with the vector to provide one or more transformed plants that express the polynucleotide; and

selecting a transformed plant that (i) exhibits a GABA concentration in non-stress conditions of up to about 0.20 milligrams GABA per gram dry weight of the plant; or (ii) does not exhibit significant loss of growth characteristics, yield, reproductive function or other morphological or agronomic characteristic compared to a non-transformed plant.

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32. The method according to claim 31, wherein the GAD enzyme comprises an amino acid sequence selected from the group consisting of the sequence set forth in SEQ ID NO: 2; the sequence set forth in SEQ ID NO: 4; the sequence set forth in SEQ ID NO: 6; the sequence set forth in SEQ ID NO: 8; the sequence set forth in SEQ ID NO: 10; the sequence set forth in SEQ ID NO: 12; the sequence set forth in SEQ ID NO: 14; the sequence set forth in SEQ ID NO: 16; the sequence set forth in SEQ ID NO: 18 and a sequence having at least about 60% identity thereto that is effective to catalyze a reaction of glutamic acid to GABA.

10 33. The method according to claim 31, wherein the GAD enzyme is a modified GAD that does not include a functional autoinhibitory calmodulin-binding domain.

15 34. The method according to claim 31, wherein the transformed plant produces GAD enzymes at a rate substantially greater than the rate at which GAD enzymes are produced by a non-transformed plant of the same species under the same conditions.

20 35. The method according to claim 31, wherein the polynucleotide is selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1; the nucleotide sequence of SEQ ID NO: 3; the nucleotide sequence of SEQ ID NO: 5; the nucleotide sequence of SEQ ID NO: 7; the nucleotide sequence of SEQ ID NO: 9; the nucleotide sequence of SEQ ID NO: 11; the nucleotide sequence of SEQ ID NO: 13; the nucleotide sequence of SEQ ID NO: 15; the nucleotide sequence of SEQ ID NO: 17 and sequences that hybridize thereto under moderately stringent conditions.

30 36. The method of Claim 31, wherein said transforming comprises:

- (i) transforming a cell, tissue or organ from a host plant with the DNA construct;

- (ii) selecting a transformed cell, cell callus, somatic embryo, or seed which contains the DNA construct;
- (iii) regenerating a whole plant from the selected transformed cell, cell callus, somatic embryo, or seed; and
- 5 (iv) selecting a regenerated whole plant that expresses the polynucleotide.

37. A transformed plant obtained according to the method of claim 31 or progeny thereof.

10 38. A plant transformed with a vector comprising a constitutive promoter operably linked to a polynucleotide that encodes a GAD enzyme, or progeny thereof;

15 wherein the plant expresses the polynucleotide; and

wherein the plant (i) exhibits a GABA concentration in non-stress conditions of up to about 0.20 milligrams GABA per gram dry weight of the plant; or (ii) does not exhibit significant loss of growth characteristics, yield, reproductive function or other morphological or agronomic characteristic compared to a non-transformed plant.

20 39. The plant according to claim 38, the plant being selected from the group consisting of duckweed, rice, wheat, barley, rye, corn, Bermuda grass, Blue grass, fescue, rapeseed, potato, carrot, sweet potato, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, bush beans, tobacco, tomato, green pepper, sorghum and sugarcane.

30 40. A non-sterile plant having incorporated into its genome a foreign DNA construct comprising a promoter operably linked to a polynucleotide that

encodes a functional plant GAD enzyme, the polynucleotide comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1; the nucleotide sequence of SEQ ID NO: 3; the nucleotide sequence of SEQ ID NO: 5; the nucleotide sequence of SEQ ID NO: 7; the  
5 nucleotide sequence of SEQ ID NO: 9; the nucleotide sequence of SEQ ID NO: 11; the nucleotide sequence of SEQ ID NO: 13; the nucleotide sequence of SEQ ID NO: 15; the nucleotide sequence of SEQ ID NO: 17 and sequences that hybridize thereto under moderately stringent conditions; wherein the plant expresses the polynucleotide.